**Introduction to Mechanobiology**
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**Week - 05**
**Lecture – 24**
**Mechanobiology of Stem Cell Fate – I**

Hello and welcome to our todays lecture of Introduction to Mechanobiology. In the last few lectures we had discussed about different modes of migration specifically focusing on 3 different aspects.

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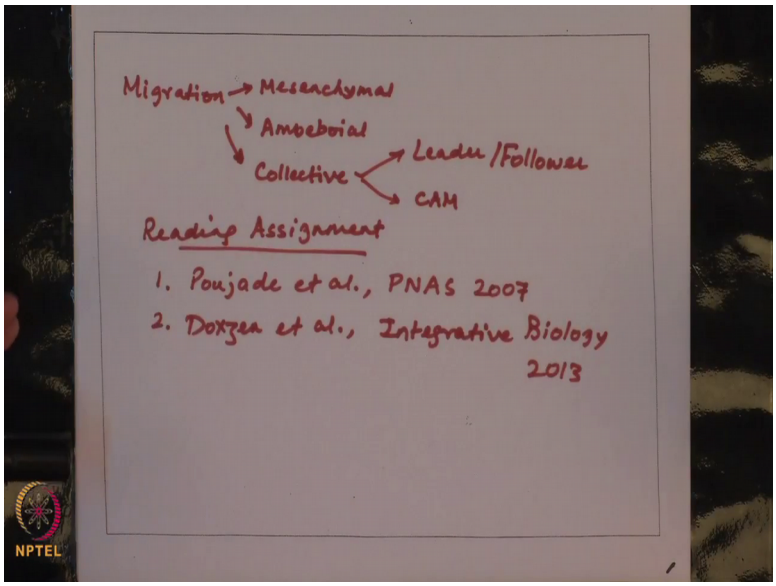


Some example of Mesenchymal Migration and how actin dynamics drives Mesenchymal Migration, one study on ambeboial migration and another one on Collective cell migration in the last lecture where I had focused on leader and follower cells and also this phenomena called coherent angular rotation when you confine epithelial cells within pattern regions.

So, as part of the reading assignment I would like to ask you refer you to these 2 papers was the PNAS paper which we discussed about the using stencils for studying, you know wound healing and also this second paper where I just touched upon about coherent angular rotation and I you know request you to go through this paper in great detail, having said that I would now switch gears and stop our discussion from migration to

some other aspects of cell behavior. Specifically I will start with behavior of stem cells or mechanobiology of stem cells and go on to mechanobiology of diseases over the next 2 weeks

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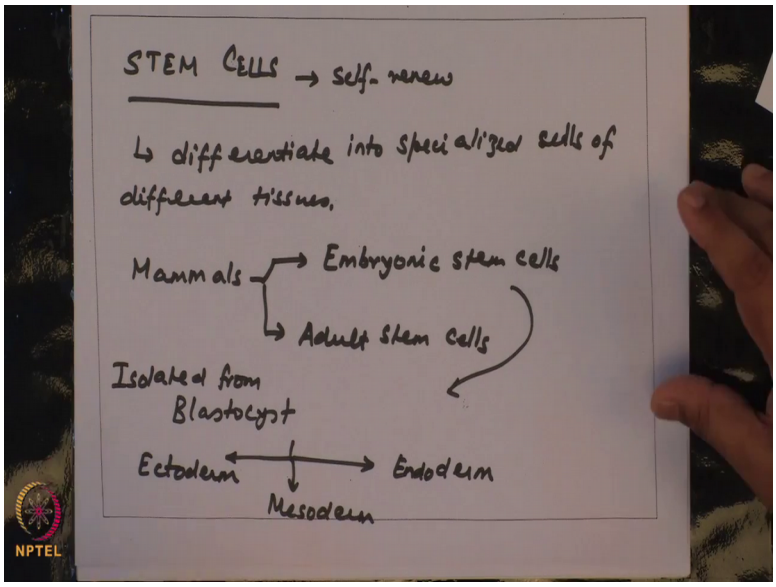


So, having said that. Today we will start discussing about mechanobiology of STEM cells, now to talk about stem cells. What are stem cells? STEM cells are undifferentiated biological cells, you have cells which can differentiate, 2 aspects of cell STEM they can self renew or divide to generate more of their own type or you can differentiate into specialized cells of different tissues. In case of Mammals you have you broadly have 2 types of STEM cells; Embryonic STEM cells and Adult STEM cells. What are escs? Escs are cells which are isolated from the Blastocysts.

So, these embryonic stem cells. These they are isolated from blastocysts and these guys are called pluripotent because they can generate cells of the Ectoderm Mesoderm and Endoderm. So, embryonic stem cells can generate all cells of all different genres, but there is some ethical issue about embryonic stem cells. So, what is the ethical issue you have these cells which are isolated from an embryo. So, by isolating cells from the blastocyst set stage you are actually killing that organism. That is the ethical issue that is the main primary problem why embryonic stem cells did not find much use for regenerative medicine applications.

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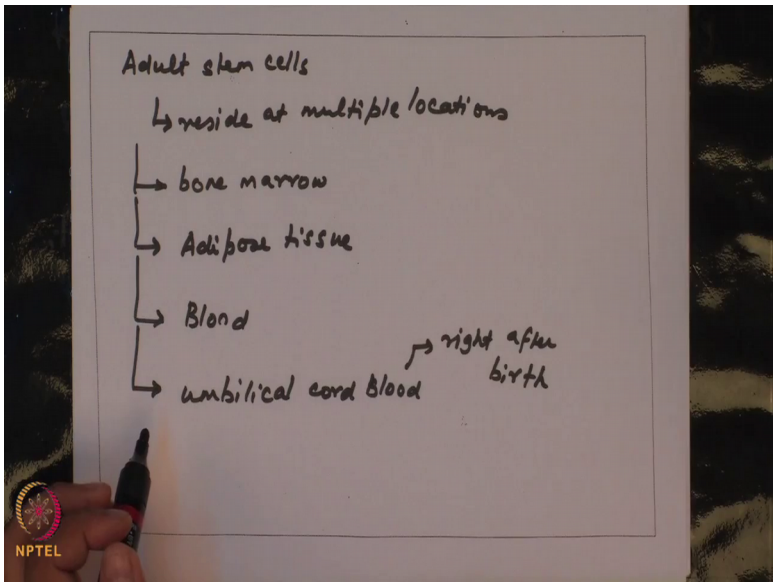


In contrast you have Adult stem cells; these stem cells reside at multiple locations and you have isolation of adult stem cells one of them from this from bone marrow, one of them from Adipose tissue, you can isolate them from Blood or you can isolate from umbilical cord this is right after birth.

So, the advantage of adult stem cells is you do not have these ethical hurdles and you can in principle. Because these cells are autoloaders, you can obtain the cells from your own body and use it either for banking. That you can use it for future surgical procedures as well as to reengineer them to differentiate them into given cell types and then re inject them back into your body. So, you want these cells would not elicit an immune response because they are from your own body. That is the promise of regenerative medicine you can use it.

If you compare the ES cells with adult stem cells. So, you can have different. ES cells ESCs are called pluripotent.

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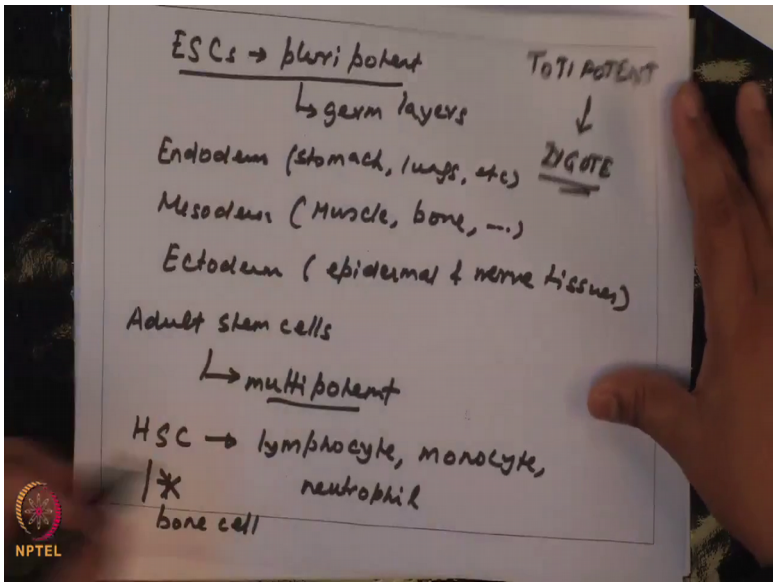


They are called pluripotent because they have the potential to differentiate into any 3 cell layers; they have the capacity to differentiate into cells of 3 germ layers. Which are Endoderm; that means stomach, lungs etcetera Mesoderm which is muscle, bone etcetera or ectoderm which include epidermal and nervous nerve tissues. ESCs are pluripotent in comparison you have adult stem cells they are what is called as multipotent.

Their ability to differentiate into all different cell types is not possible. For example, if you have a hematopoietic stem cell h is for hematopoietic stem cell. So, it can give rise to all cells blood cell types; including lymphocyte, monocyte and neutrophil. But this guy it cannot it cannot give rise to a bone cell. So, this is called a multipotent and in comparison to pluripotent or multipotent you have one more case which is called Totipotent and example is the ZYGOTE. These can also give rise to your sponsor. So, this is totipotency. In addition float, the way the stem cell hierarchy was initially imaged in people would always think that from this stem cell you give rise you have these cycles, this guy can self renew or differentiate.

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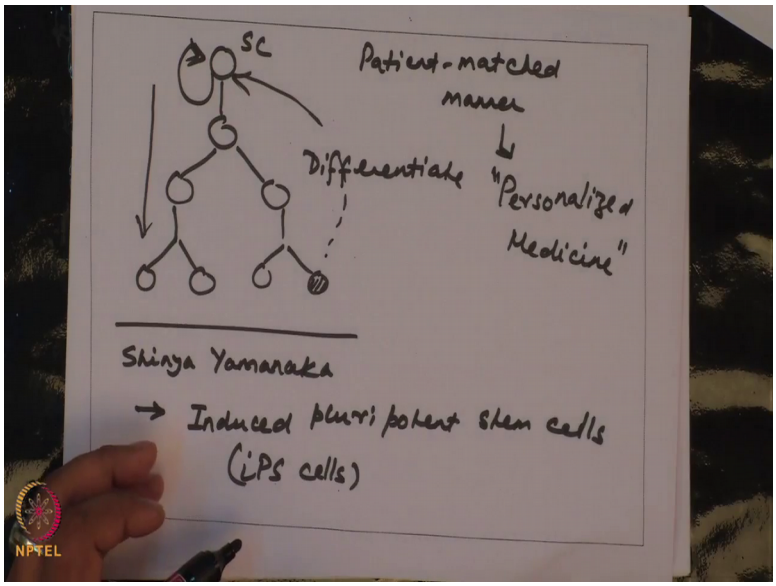


Suggesting that you can only traverse the tree in this direction or downward direction, but this was changed by the seminal work of Yamanaka Shinya Yamanaka very recently. So, he showed that you can generate Induced pluripotent stem cells in short they are referred to as iPS cells.

What iPS cells are they are reprogram, you can take a terminate differentiated cell like a 5 blast and you can reprogram it to generate the original stem cell. These are reprogrammed where you the mature cells are reprogrammed to become pluripotent. What are the strengths of this iPS technology, because you can you can in principle iPS cells you can derive from adult tissues. So, not only does it bypass the need for embryos, but you can be this can be made in a patient matched manner. So, in what does this mean that each patient can generate his her own pluripotent stem line same cell line and this is what is the beauty of personalized medicine. The other interesting aspect of iPS series you can simulate diseases which take long term to manifest and cannot be studied in vitro using ipss, but still they are far from that stage you can think of therapeutically engineering iPS cells for personalized medicine.

So, this is just a broad introduction to stem cells, now typically if you look at all these cells these different cell types a muscle cell is more elongated.

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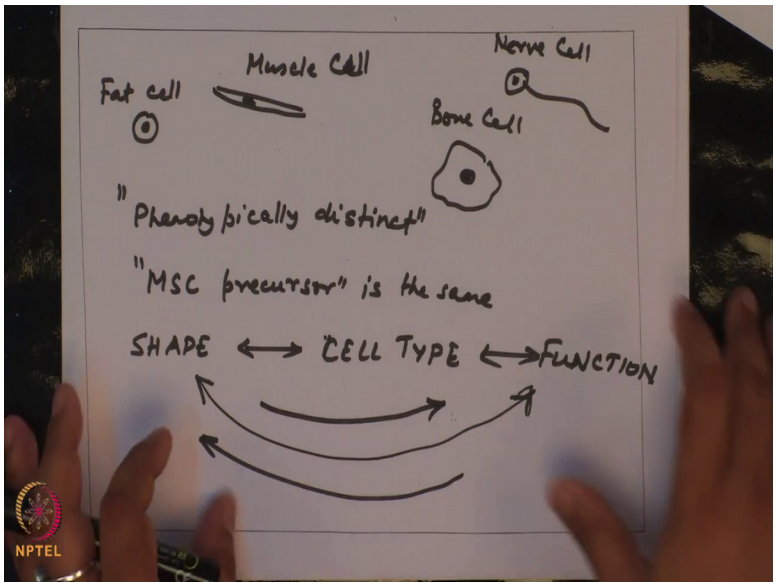


A Fat cell this is the shape of a Fat cell, a Muscle cell, and nerve cell, versus a bone cell is you have the nuclei at the center. What you see that these cells have distinct phenotype they are “Pheno typically distinct”. Though descend from a common MSC you know you have MSC from which they originate. So, mesenchymal stem cells precursor is the same is the same you have distinct phenotypes for each of these cells.

In other words shape seems to be correlated to cell type and each of these cell types has a given function. So, the question here then becomes that is it possible that function of cells is directly related to the shape and what is the nature of the dependency is it that shape determines function or function determines shape. That is the question to be asked. So, typically we always think that genotype dictates phenotype, but; however, is it possible that by changing the phenotype you can alter the function of stem cells.

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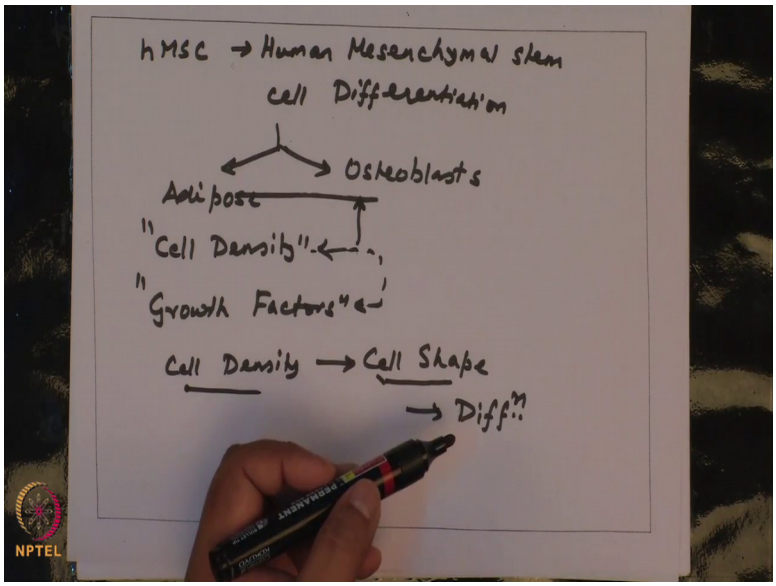


We will discuss one such paper in the context of hMSC, hMSC differentiation human mesenchymal stem cell.

These guys are known to differentiate into adipose or fat cells as well as osteoblast or bone cells. So, now one of the striking observations in these experiments is that the decision to differentiate into an adipose cell versus an osteoblast is of 10 dependent on the “Cell Density”. The density at which these cells are plated seems to have a strong correlation in the lineage to be which these cells differentiate. Of course, one would imagine that “Growth Factors” at the only things which determine the overall response. But cell density what is the correlation between cell density and growth factors in relation to this differentiation is it possible. So, imagine this picture that cell density actually impacts the cell shape and cell shape then impacts the differentiation status.

We are saying that it is the phenotype. So, you are kind of indirectly controlling phenotype by cell density and then this phenotype is what dictates this overall response.

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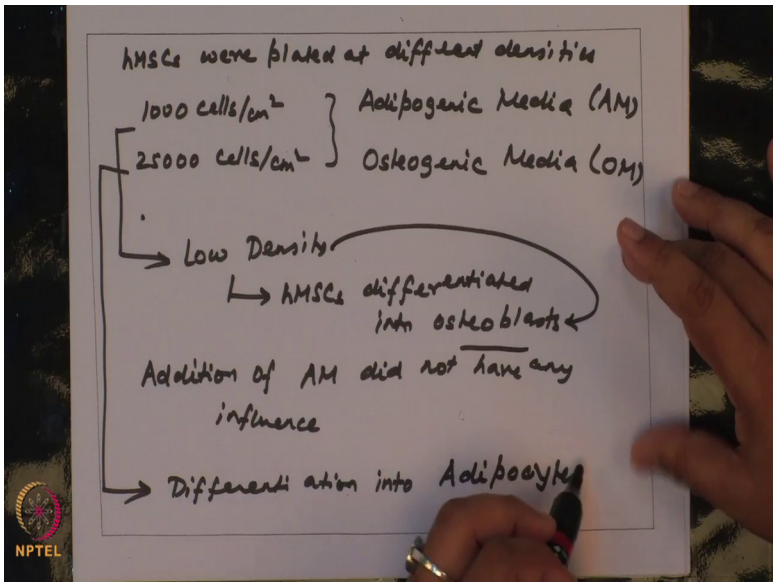


What the author started off was they did this experiments in which you cultured cells hMSCS where plated at different densities either at 1000 cells per centimeter square or 25000 times higher 25000 cells per centimeter square. And then they also added one more factor which is your soluble queue or growth factor they also added did these experiments in the context of Media, which would typically induce differentiation of hMSCs in adipocytes or Osteogenic Media where the chemical factors present in this media induces osteogenic differentiation.

What they found was when they plated cells at this low density all the cells hMSCs differentiated into osteoblasts. And even addition of media did not have any influence, in contrast at the cell seeding density of 25000 square centimeter square you have differentiation into adipocytes. What you find is as a function of time at low density you get osteoblasts, low density leads to osteoblasts, high density a high density leads to adipocytes. So, suggesting that again demonstrating that cell plating density influences with cell fate.

If we are to look at our argument of cell density in directly impacting the cell ship, so one of the things what you are doing is in a given area.

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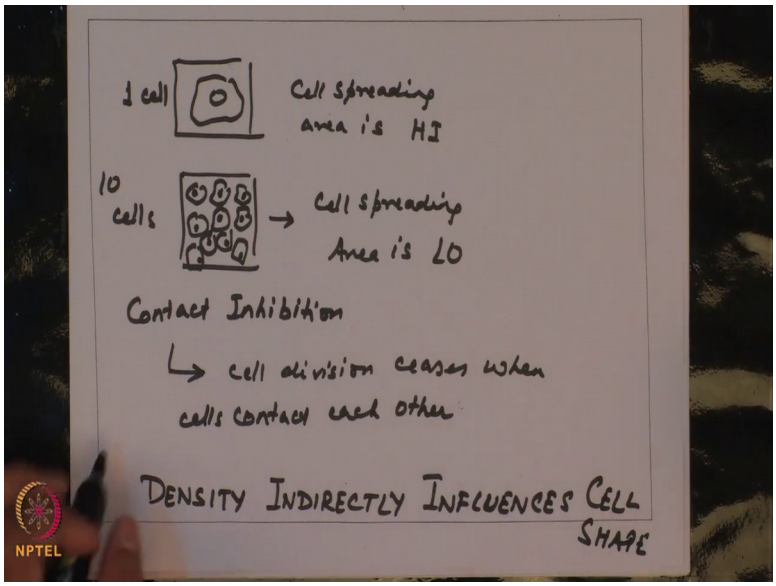


Let us say in this given box plate a single cell versus this is one cell plated in this was box versus stem cells plated in this box simultaneously. What you see that one of the indirect consequences of plating cells that a high density would mean that the allow will area for a single cell to spread is lot less. So and also first of all your cells spreading area is low here and here cells spreading area is high; is high also you have this phenomena called Contact Inhibition and in this phenomena you have cell division ceases when cells contact each other.

So, in a sense what you see here what this shows you is that density indirectly influences cell shape. Is it possible that it is this cell shape or cell size which determines the nature of the differentiation try at there so; however, this particular experiment it is difficult to answer that question because it can very well be possible that when you have high density, the growth factors, other things that they the cells secrete might have a role to play, also the cells touching each other might also have a role to play in this differentiation.

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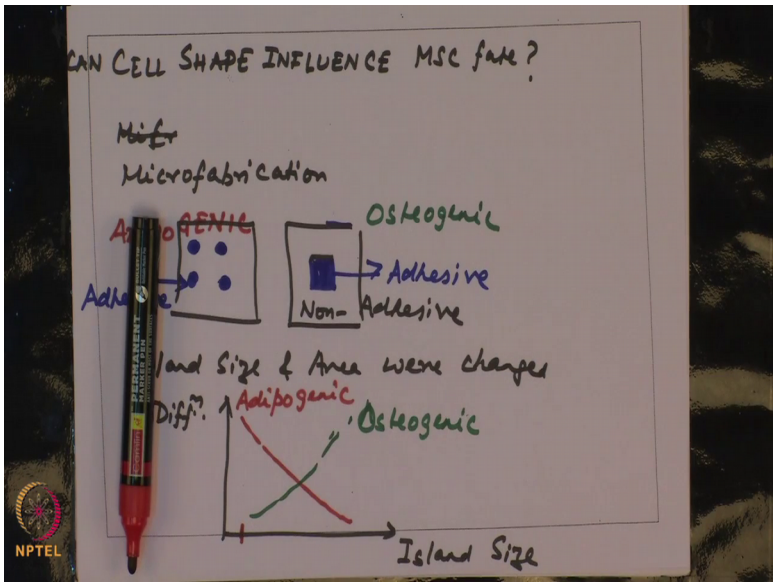


So, to ask if cell shape alone, the question is can Cell Shape; can Cell Shape influence MSC fate it is a question.

To ask that question what is the authors did was dip again using Micro fabrication. So, used mic they used micro fabrication and what you have in micro fabrication in a background of surface you can pattern. So, you have seen what is micro fabrication earlier in the context of focal adhesions you can pattern these islands either with very small dots or you can have let us say, you can make this square patterns with bigger area. These areas are adhesive and this is Adhesive and this area is Non-Adhesive. What they changed was the area of the Island. Island area was Island size and area was changed. So, what they found was when they varied Island size, this is island size and let us say it is the y axis is the extent of differentiation.

What you see what the authors found was with increase in Island size, they observe the following thing this is Osteogenic and this is; increase in Island size switched the differentiation status with a more prominent differentiation on small Island size is. This environment will be more and this environment will be more Osteogenic. So, bigger the Island size more Osteogenic differentiation less Adipogenic differentiation, smaller the Island size more Adipogenic differentiation less Osteogenic differentiation.

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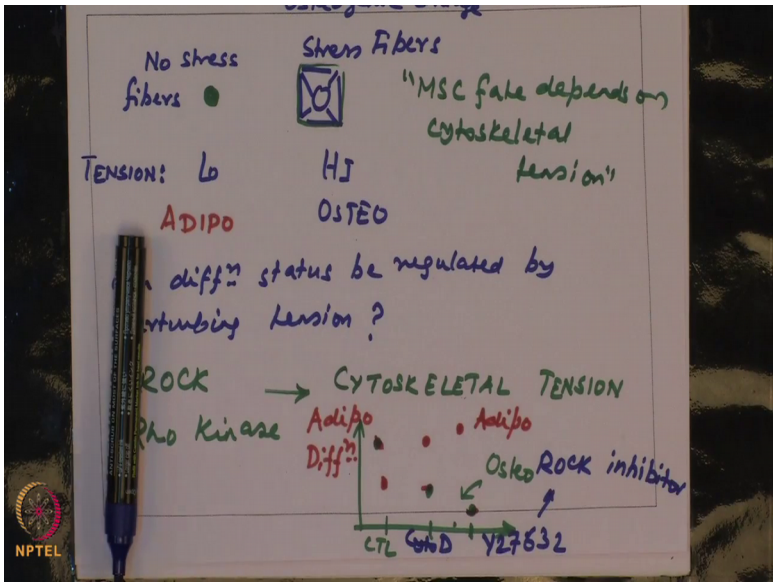


Now, when they looked at the cells, if they look at the cells you had a strong stress fibers in case of cells differentiate into osteogenic lineage and in case of Adipogenic in small Islands. So, there was No stress fiber. Again going back to the analogy of cell as a tenth greater amount of stress fibers, if would to draw tension the level of tens cytoskeletal tension this is low, this is high. So, again you have this correlation between tension and the lineaged differentiation. In Adipogenic in low and tension is low you have Adipogenic differentiation, when tension is high you have Osteogenic differentiation.

They then asked that can differentiation status be regulated by perturbing tension. It is the question that they asked and what they found was again. So, the perturb tension using, you have ROCK Rho Kinase is one of the signaling pathways which directly mediates Cytoskeletal Tension. What they found compared to control if the added 2 drops CYTO D or Y 2 7 6 3 2 Y 2 7 6 3 2 is the ROCK inhibitor and CYTO D is an actin depolymerizing is it. Once again Adipogenic differentiation dropped, this is my adipo differentiation.

So, you lower the tension sorry. So, sorry this yeah. So, you are this is my no. This is OSTEO differentiation and this is Adipo differentiation. Lower the tension if you lower the tension your osteogenic differentiation drops, if you load the tension then you are Adipogenic differentiation increases. These results show that MSC fate depends on cytoskeletal tension.

So, with that I will stop here, to summarize what we discuss today we showed that, in case of stem cells, in mesenchymal stem cells by perturbing things like shape or tension you can perturb it is differentiation perturb or pathways, with more amount of tension leading to an Ostegenic differentiation and lowering of tension leading to Adipogenic differentiation and this changing of tension is also associated with changing of the cell shape. So, in case of low tension the cells are more rounded do not have much cytoskeleton verses in when they are highly tensed you have structures which are more elongated most you know a stretched out configurations of the cell.

Thank you for your attention.